Effect of Linker Composition on Efficiency in Cellular Selection

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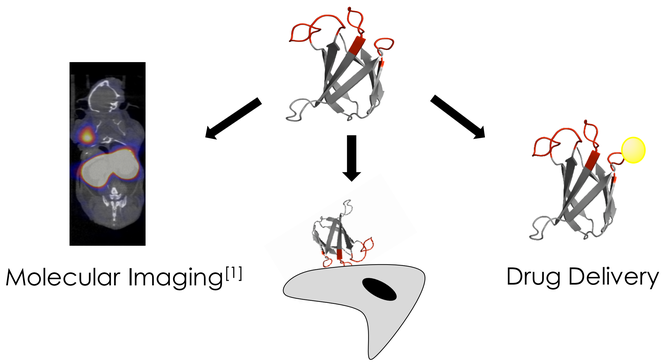
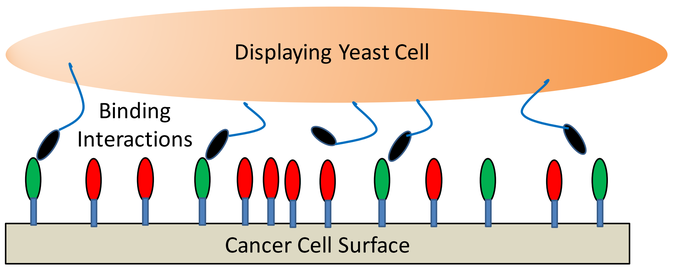
Background

Motivation:
Engineered protein ligands with specific binding to targets are desirable for a number of applications.



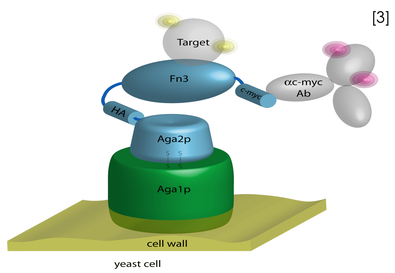
Existing Problem:
The inability of recombinant protein to recapitulate true cellular protein hinders the discovery of synthetic binding ligands. Prior work has shown the ability of an extended yeast surface display linker to allow recovery of high-affinity binding ligands through selection on target-expressing mammalian cells[2]. However, the optimum length and composition of these linkers has not been rigorously quantified.



Hypothesis:
1. Longer display linker will increase recovery of weaker binders, by improving the accessibility of ligands to receptors.

2. Hybrid Rigid/Flexible increases recovery of weaker binders against mammalian targets with lower expression.

[3]

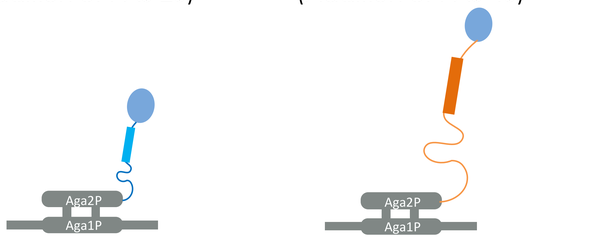


The Four Designs

Four different linker designs were tested in an EGFR-binding model system to determine the optimal display linker for further study. Two designs added flexible linker length by increasing the length by 50% and 100% respectively. Another designs added a hybrid rigid/flexible linker to the existing flexible display linker to test whether rigidity further improves the accessibility of ligand to the target molecule.

Extended Flexible Linker (Addition of PAS 20)

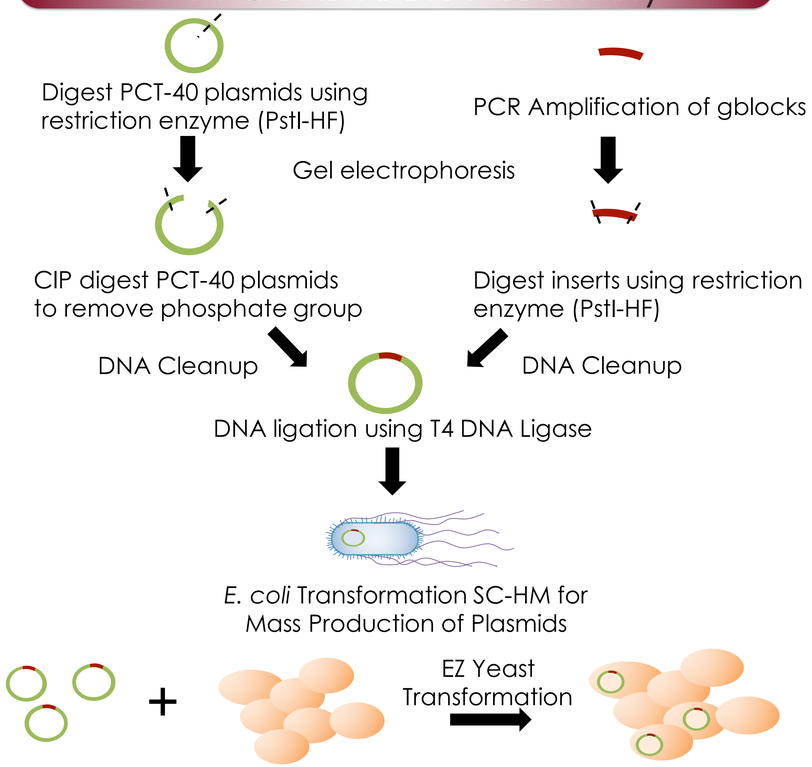
Extended Flexible Linker (Addition of PAS 40)



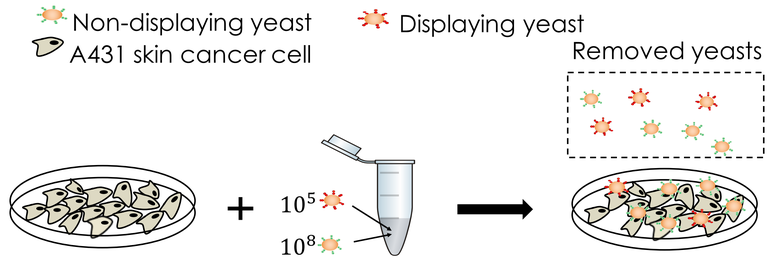
Hybrid Flexible/ Rigid Linker (Addition of EAAAK 20)

Hybrid Flexible/ Rigid Linker (Addition of EAAAK 40)

DNA Constructs Assembly

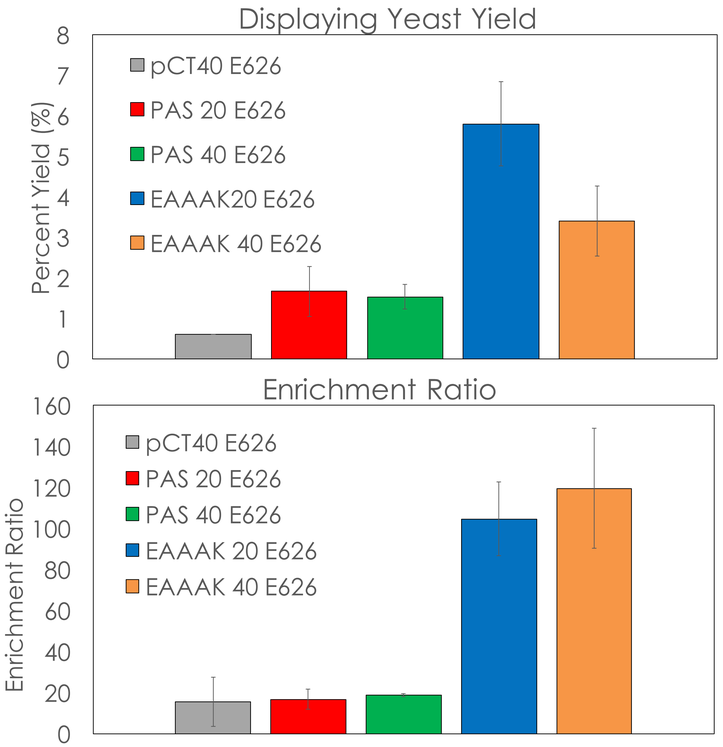


Cell Panning



Results

Introduction of rigid linkers (EAAAK sequences) have resulted in five times higher in enrichment ratio and yield, while the introduction of flexible linkers (extended PAS sequences) does not show significant improvement.



Future Work

- Attempt to recover lower affinity ligands (e.g. PCT 40 E.6.2.6' AASV) in scenarios where existing linkers have previously failed.
- Construct PAS 600 and EAAAK 600 for both PCT 40 E.6.2.6' and AASV and recover on A431 cells.

Acknowledgements

References: [1] Hackel, B. et al. *Radiology*. 2012 [2] Stern, L. A. *et al.* ,2016 [3] Hackel, B. J., & Wittrup K. D., 2016